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Viral-induced neurodegenerative disease

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Viral etiology has been postulated in a variety of neurological diseases in humans, including multiple sclerosis. Several experimental animal models of viral-induced neurodegenerative disease provide insight into potential host- and pathogen-dependent mechanisms involved in the disease process. Two such mouse models are the Theiler's murine encephalomyelitis virus (TMEV) infection and mouse hepatitis virus (MHV) infection.

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Abbreviations

AG	aminoguanidine
CNS	central nervous system
EAE	experimental allergic encephalomyelitis
IFN	interferon
IL	interleukin
MCP-1	monocyte chemoattractant protein-1
MHV	mouse hepatitis virus
MS	multiple sclerosis
NO	nitric oxide
NOS	NO synthase
TMEV	Theiler's murine encephalomyelitis virus
TNF	tumor necrosis factor

Introduction

Prevalent hypotheses to explain the etiology of multiple sclerosis (MS) suggest that an infectious agent encountered during adolescence might prime a disease process that only appears in the adult after a variable period of quiescence [1]. Efforts to identify a causative agent of MS have focused on finding a single virus causally linked to MS; however, it is clear from a variety of experimental model systems that many viruses are capable of inducing demyelinating syndromes (Table 1). Because of their obvious phylogenetic diversity, these agents are unlikely to share a single common mechanism of induction of disease. It seems more likely in our opinion that the host response to a viral infection of the central nervous system (CNS) is the common thread that ties together these seemingly disparate infections. Consideration of the mechanism of induction of demyelinating disease must consider several mutually non-exclusive alternative mechanisms as summarized in Table 2. Moreover, the pathogenic response to a viral infection might resemble in many aspects the effector mechanisms observed in the autoimmune model, experimental allergic encephalomyelitis (EAE). A fundamental difference in the viral and EAE models is that of the infections etiology; infection is a necessary requirement of demyelination in the viral models, and it is precisely this cause–effect relationship that makes these viral models attractive platforms to explore the etiology and pathogenesis of demyelinating disease.

Table 1

Viruses associated with demyelinating disease.

Virus	Family	Host
Mouse hepatitis virus	Coronavirus	Mouse, rat
Theiler's virus	Picornavirus	Mouse
Measles virus	Paramyxovirus	Man
Canine distemper	Paramyxovirus	Dog
Semliki forest virus	Alphavirus	Mouse
Visna	Lentivirus	Sheep
HTLV-1	Lentivirus	Man
HIV	Lentivirus	Man
Marck's disease virus	Herpesvirus	Turkey
HSV-1	Herpesvirus	Man
JC virus	Papopavirus	Man

Table 2

Potential mechanisms of demyelination induced by MHV.

Potential mechanism	Examples	References
Virus persistence	Disrupts oligodendrocyte function	[37]
	In astrocytes, results in chronic iNOS, TNF-α and IL-6 expression and glial toxicity	[14]
	Persistent viral antigens expressed in glial cells target chronic immune response to white matter	[23••,24••]
Molecular mimicry	Virus infection primes immune response to cross-reactive myelin antigens such as MBP or PLP	[36,38]

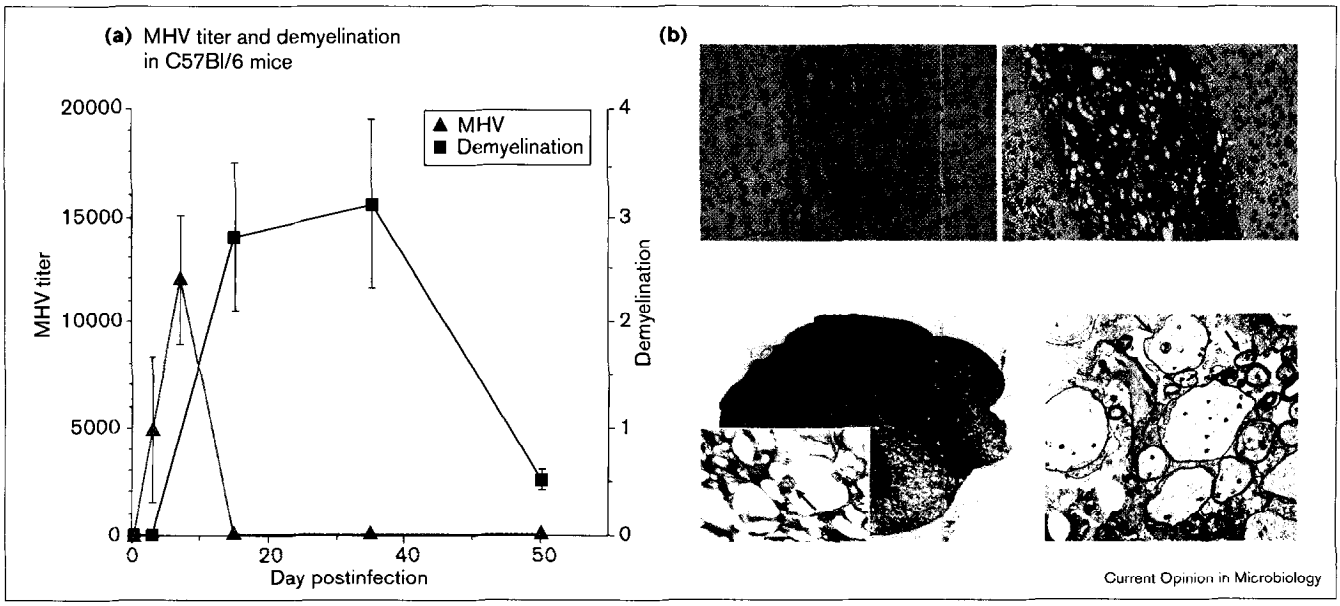
iNOS, inducible NOS; MBP, myelin basic protein; PLP, proteolipid protein.

This brief review emphasizes recent advancements that have contributed to our understanding of how viruses contribute to developing neuropathology following infection of the CNS. Viral-CNS disease models are numerous and complex, however, for this discussion we have focused our attention upon two of the most widely used models of virus-induced demyelination: Theiler's murine encephalomyelitis virus and mouse hepatitis virus.

Viral models of demyelinating disease

The mouse hepatitis virus (MHV) model provides a unique probe that has proven useful in interpretation of demyelinating disease. Coronaviruses are an ubiquitous group of positive stranded RNA viral pathogens of man and animals

Figure 1



Infection of adult mice with the MHV-JHM virus. **(a)** Time course of viral infection and demyelination following infection with a demyelinating variant of MHV-JHM. Free virus is evident for only 10–15 days post-infection; however, demyelination persists for greater than 50 days. **(b)** Foci of demyelination are characterized by the co-localization of viral RNA (top left) and demyelinating white matter lesions (top right). Late lesions show extensive demyelination active macrophage phagocytosis of myelin debris (bottom left, arrow) and the presence of demyelinating axons (bottom right, arrows).

associated with a wide spectrum of respiratory, gastrointestinal and neurological diseases. Infection of adult mice with the MHV-JHM strain of the virus elicits a reproducible syndrome characterized by an early peak of virus replication in the brain, followed by an acute T-cell-dependent demyelinating encephalomyelitis involving both brain and spinal cord (Figure 1). Active viral replication is controlled early in the MHV model: by 10–15 days after infection viral titers have dropped below the limit of detection, although viral RNA persists in white matter for several months. Notable is the uncoupling of early viral replication and later induction of demyelination. This pattern is in sharp contrast to the Theiler's murine encephalomyelitis virus (TMEV) model in which infection is followed by a productive persistent infection of white matter. These viral models allow investigation into the details of the virus–host interaction that lead to disease.

Nitric oxide and CNS disease

The gaseous free-radical nitric oxide (NO) is generated through the action of one of three isoforms of the enzyme nitric oxide synthase (NOS). NOS1 and NOS3 are expressed constitutively resulting in low-level output of NO [2]. These two enzymes function primarily in the maintenance of tissue homeostasis, as well as vasoregulation. In contrast, NOS2 (inducible NOS or iNOS) transcripts are expressed following exposure of a wide variety of cells to cytokines or bacterial endotoxin. Therefore, under proinflammatory conditions arising from either microbial infection and/or injury, NOS2 expression is often detected and considered an important contributor to the pathogenesis of a wide variety of inflammatory diseases.

The CNS is not exempt from expression of NOS2. High-level expression of the gene encoding NOS2 has been detected in numerous neuropathologies including MS [3] and Alzheimer's disease [4]. Furthermore, NOS2 has been detected within the CNS following infection with a wide variety of both DNA and RNA viruses [5]. Within the CNS, astrocytes and microglia/macrophages are the predominant cellular sources of NOS2 [6,7]. Although the contributions of NO generated by NOS2 to CNS damage are not fully understood, studies have demonstrated that NO exerts a cytotoxic effect on cultured oligodendrocytes suggesting that this free-radical might also contribute to demyelination in patients with MS [8]. In addition to a direct cytotoxic effect, NO can interact with superoxide anion (O_2^-) to form peroxynitrate anion, which is a strong oxidant that might contribute to pathology of neurodegenerative diseases [9•,10].

NOS2 is detected within brains and spinal cords of mice during both acute and chronic stages of infection with both TMEV and MHV [6,7,11,12•,13,14]. Astrocytes and cells of the monocyte/macrophage lineage were found to express the NOS2 gene in both viral infections [6,7,12•]. Within TMEV-infected mice, both astrocytes and macrophage/monocytes stained positive for NOS2 during acute and chronic stages of disease. In contrast, within MHV-infected mice the cellular source of NOS2 was

dependent upon the stage of disease. During the acute meningoencephalitis period (5–7 days post-infections), inflammatory macrophages were the predominant source of NOS2 transcripts and protein, while astrocytes almost exclusively expressed NOS2 during the chronic demyelinating stages of disease [6,7]. These observations suggest that the stage of disease (i.e. acute versus chronic) might dictate the contributions of NO to the disease process.

Administration of aminoguanidine (AG — a selective inhibitor of NOS2 activity) to either TMEV- or MHV-infected mice resulted in a marked reduction in the severity of clinical disease, as well as inflammation and demyelination, suggesting a role for NOS2 generated NO in the pathologies of these CNS infections [14]. This AG treatment did not affect viral replication or the ability of the animals to clear virus indicating that the reduction in the severity of disease was due to inhibition of NOS2 activity rather than an indirect effect. The mechanism(s) by which NO might contribute to these diseases is unknown. In addition to its well demonstrated cytotoxic effects (alone or in combination with O_2^-), NOS2 generated NO may contribute to neurologic disease in a subtler manner. Administration of AG to MHV-infected mice was found to result in a selective decrease in the level of mRNA transcripts for monocyte chemoattractant protein-1 (MCP-1), which is a C-C chemokine that is thought to function in attracting both T cells and macrophages during inflammatory conditions [15•,16]. Therefore, it is possible that NO generated by NOS2 might contribute to CNS disease by promoting transcription of pro-inflammatory factors.

Cytokines/chemokines and CNS disease

CNS infection with a wide variety of different viruses results in increased local expression of both cytokines and chemokines. Production of these soluble mediators by activated T cells, macrophages and resident glia (e.g. astrocytes, oligodendrocytes and microglia) allows for a variety of responses including the initiation and maintenance of an inflammatory response through induction of adhesion molecule expression by endothelial cells, and increased expression of cytokine and chemokine receptors by infiltrating cells. Furthermore, production of these factors allows for cell-to-cell communication that might be important in controlling cell proliferation, antigen presentation, and production of potentially toxic products [17,18].

Both MHV and TMEV induce expression of cytokine and chemokine genes following infection of the mouse CNS. Cytokine genes that are expressed during acute MHV infection (between days 5–7 post-infections) of the CNS include interferons alpha, beta, and gamma (IFN-α, β, γ), tumor necrosis factor-alpha (TNF-α), interleukins 1 alpha and beta, (IL-1α and β), IL-2, IL-6, and IL-12 [6,19,20]. Although this profile indicates a T helper cell type 1 (Th1) profile is prevalent during infection, mRNA transcripts for IL-4 and IL-10 can be detected suggesting that a polarized Th1 or Th2 response is occurring. Furthermore, the severity of infection does not appear to dictate the cytokine response within the CNS. In other words, similar cytokine profiles are obtained when the highly neurovirulent MHV-JHM or attenuated strains are used for experimental infection. Similarly, CNS infection by TMEV results in a predominantly Th1 cytokine profile (e.g. IFN-γ, TNF-α, and IL-2) that did not appear to be strain dependent [21].

In addition to cytokines, members of both the C-C and C-X-C family of chemokines [22] are also expressed in the brains and spinal cords of MHV-infected mice; for example, the C-X-C chemokines genes encoding the cytokine response gene-2/interferon inducible protein-10 (CRG-2/IP-10), MIG, and macrophage inflammatory protein-2 (MIP-2) are up-regulated, and the C-C chemokines genes encoding MCP-1, MCP-3, MIP-1β and RANTES are also up-regulated [23••]. Although the role of these molecules in the pathogenesis of either the acute stage or the chronic stage of disease is unclear, it is conceivable that these molecules function to attract inflammatory leukocytes into the CNS following viral infection. These inflammatory leukocytes then contribute to host defense by eliminating virus.

Mechanisms of host defense against MHV infection of the CNS include the generation of perforin-dependent virus-specific cytotoxic T lymphocytes (CTLs) [24••]. Cytokines might also aid in host defense against MHV by contributing to the elimination of virus from the CNS. Recent studies demonstrate that IFN-γ knock-out mice display higher mortality and increased viral burden within the CNS compared with age-matched control mice [13]. Interestingly, the antiviral effect of IFN-γ is dependent upon the host cells infected: IFN-γ is not required for clearance of virus from neurons, but is of critical importance in clearance from oligodendrocytes [24••]. This is interesting in that these observations suggest separate, distinct mechanisms exist within the CNS that contribute to viral clearance.

In addition to their potential role in antiviral immunity, cytokines and chemokines might also contribute to CNS pathology in animals persistently infected with MHV. Chronic expression of these factors may ultimately lead to deleterious effects by triggering the chronic release of toxic molecules such as NO. Cytokines that are expressed in the spinal cords of mice persistently infected with MHV include TNF-α, IL-6, and IL-1β [6]. These cytokines were localized primarily to white matter tracts associated with demyelination and MHV infection, and were produced predominantly by astrocytes. The majority of cytokine-expressing astrocytes, however, were not infected with MHV suggesting that activation and cytokine secretion is not due to viral infection.

A recent study reports that the anti-inflammatory cytokine IL-10 has no substantial role in contributing to either viral clearance or demyelination in MHV-infected mice. A subset of IL-10–/– mice, however, did exhibit a moderate increase in the number of inflammatory mononuclear cells

compared with control mice, suggesting that IL-10 may exert some minor control of inflammation during acute stages of disease [25].

Individually, cytokines might contribute to demyelination by increasing vascular permeability, sustaining T cell responses, or increasing the expression of adhesion molecules [14,18]. Alternatively, these cytokines might work in concert with one another to enhance the inflammatory response, as well as to activate cells to express cytotoxic molecules. Although TNF-α has been postulated to contribute to demyelination in both EAE and patients with MS, passive administration of neutralizing antibodies to TNF-α did not diminish MHV-induced demyelination indicating that TNF-α does not play a dominant role in this infection [26].

The chemokines CRG-2/IP-10 and RANTES are expressed most abundantly in the spinal cords of chronically infected mice with demyelination [23••]. Similar to what has been reported with cytokines, expression of these chemokines was localized to areas of virus persistence and demyelination. CRG-2/IP-10 was produced predominantly by uninfected astrocytes. *In vitro* studies have also demonstrated that MHV infection of astrocytes, as well as brain endothelial cells, results in cytokine and chemokine gene expression [23••,27]. Further characterization of the role(s) of these multipotent factors in MHV-induced demyelination remains to be determined. Recent reports [22,28••], however, have suggested a potential role for RANTES in the pathogenesis of MS since inflammatory leukocytes have been reported to be present within MS plaque lesions. Whether RANTES has a role in contributing to virus-induced demyelinating disease has yet to be determined.

T cells and demyelination

One theory for the underlying pathological mechanism in MS patients is that activated, myelin-specific CD4+ T cells secrete cytokines and chemokines, which function to attract inflammatory cells into the CNS and/or activate inflammatory cells and resident glia to produce factors that might be toxic to oligodendrocytes. Studies from animal models support the idea that T cells are important contributors to the demyelination [29]. For example, in the well characterized autoimmune demyelination model, EAE, demyelination has been shown to be mediated by neuroantigen-specific major histocompatibility complex (MHC) class II restricted T cells [29,30].

T lymphocytes (both CD4+ and CD8+ subsets) have been shown to contribute to the pathogenesis of TMEV induced demyelination. A recent report by Murray *et al.* [31•] has demonstrated that CD4+ and CD8+ T cells have distinct, nonredundant roles contributing to host defense and chronic demyelination disease following TMEV infection of the mouse CNS. The severity, however, of neurologic disease and demyelination was markedly enhanced in mice lacking CD4 antigen (CD4–/–) compared with CD8–/– or control mice suggesting that these cells might exert a protective effect during the course of disease.

The contribution of T cells to demyelination in MHV-infected mice is unclear and remains controversial. Although studies have reported that T cells are essential for demyelination, other work has shown that demyelination occurs when either CD4+ or CD8+ T cells are depleted [32–35]. The authors [34], however, state that their experimental results do not exclude the possibility that T cells might be important in initiating demyelination early in the infection. Houtman and Fleming [33] have reported that a percentage of either $A_{\alpha\beta}$ ko mice (lacking MHC class II and have low numbers of CD4+ T cells) or β2 microglobulin ko mice (lacking MHC class I and have low numbers of CD8+ T cells) developed demyelination following infection with the MHV-JHM strain indicating that neither T cell subset is required for demyelination to occur.

Conclusions

It appears evident to us that continued presence of a viral genetic signal as either a replicating virus (i.e. in TMEV infection) or a persistent viral RNA and protein (i.e. in MHV infection) serves to drive the chronic inflammatory response in these models. Following TMEV infection anti-self reactivity has been observed as epitope spreading; reactivity with self antigens expressed on proteolipid protein (PLP) of myelin [36]. This anti-self reactivity might serve to amplify the intensity of white matter damage. However, a parallel anti-self reactivity has not been reproducibly described in the MHV model, and, therefore, it does not appear to be requisite for demyelination. Moreover, the MHV model presents an interesting challenge in answering the central question concerning what are the mechanisms of viral RNA persistent in the absence of viral replication. This paradigm would appear to fit the observation in MS of an early infection linked to later disease and influenced by genetically determined host response.

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